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Research Article

EXPLORATION OF MUCIN PROTEIN FROM *MACROCHLYMUS INDICA* AND DETERMINED ITS IMMUNOGENICITY STUDIES

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ABSTRACT

Introduction – Mucins are included under the category of high molecular weight (i.e. *O*-glycosylated) proteins which plays a crucial role with respect to protection, lubrication and acid resistance of the epithelial cell surface. In an effort to examine its mucin protein extracted from abdominal region of snail mucus i.e. *Macrochlymus indica* and determined its immunogenicity potential against rubella vaccine antigen.

Methods- For these studies, we determined the protein content from snail mucus (*Macrochlymus indica*) using Nanodrop method. In addition, indirect ELISA was performed using rubella vaccine (live attenuated vaccine) as coating antigen and determined its antibody (IgG) titre using variable doses of mucin protein and also determined its total cellular content including proliferation rate in human whole blood against rubella vaccine antigen.

Results- The results showed that mucin protein showed rapid enhancement in antibody (IgG)titre at lower doses(624 µg) but also showed some enhancement with respect to total cellular content including proliferation rate (312 µg and 624 µg) as compared to control and standard i.e. rubella vaccine.

Conclusion-Overall, this study suggests that mucin protein of *Macrochlymus indica* showed immunogenic potential against rubella vaccine antigen.

Keywords: Immunogenicity; Macrochlymus indica; mucin; Nanodrop; ELISA

INTRODUCTION:

Vaccine, biological preparation that improves immunity against particular pathogen or disease. Normally, vaccine typically contains an agent that resembles a disease causing microorganism, and is often made from weakened or killed form of the microbe, its toxins or one of its surface proteins [1, 2]. In general, vaccines may be prophylactic or therapeutic and is able tostimulate the body's immune system and is able to recognize and eliminated infectious agent from our immune system [1-3].

Snails belongs to theKingdom Animalia, class Gastropoda [4]. Gastropods are able to adapt to a variety of living conditions and they do not require large amounts of food. Gastropods belong to phylum Mollusca, classification of invertebrate animals with soft unsegmented body, sometime covered with a shell which is made up of calcium carbonate [5, 6]. Snails move by gliding along with their muscular foot, lubricated with mucus and it is covered with epithelial cilia. They secrete mucus externally to keep their soft bodies from drying out. Snails are hermaphrodites, means that they have the reproductive organs of both sexes on them. The average life span of a snail is approx. 10 to 15 years. There are 1488 species of land gastropods (species of snails & slugs) [4-7].

Mucus is a viscous, slimy mixture of glycoprotein such as mucins, water, immunoglobulins inorganic salts etc. A major function of mucus is to protect against infectious agents such as fungus, bacteria and viruses. Mucin are a diverse family of densely glycosylated proteins [8, 9].Mucin domains within the protein core are totally rich in amino acids (i.e.

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threonine, serine and hydroxyproline) enabling post-translational O-glycosylation. In view of its high glycosylated properties of mucins make them resistant to proteolysis andable to hold water molecules and showed gel like properties which is reported in mucosal barriers. Mucins also contain cysteine-rich region that precipitate in intermolecular cross-linking and are typically secreted as large aggregates. Mucins may also associated with membranes and may serve as receptor like ligands for carbohydrates- binding molecules [10-13].

Macrochlymus indica is considered to be one of the most potent species of Mollusc which belongs to the family gastropoda and is considered as most serious threat as pest that totally affect negatively on agriculture including natural ecosystems and human health [14]. This species is commonly found in leaves and flowers of wild plants. In the present study, we focused on mucin protein extracted from the abdominal region of*Macrochlymus indica* and determined its immunogenicity against specific protein antigen.

MATERIALS AND METHODS

Distribution, morphology and shell distribution

Macrochlymus indica is nocturnal, hidden in soil and normally feed on the leaves of beans, cabbage, cauliflower and some wild including ornamental plants for food. This organism is elongated and purplish grey in color. In general, small right shell-lobe and left is narrowly communicated over the edge of the peristome and its basal side gives off a short tongue-like process. In Macrochlymus indica,right dorsal lobe is very narrow and it elongates the left portion in two distinct parts. Overall, shell diameter of *Macrochlymus indica* [14] ranging from approximately 18 to 20 mm and its width (16-18.5 mm),height (8.5 mm)

Collection of mucin protein

In Macrochlymus indica, prick abdominal region for mucus collection using syringe needle. Mucus in the form of liquid which is viscous and sticky were collected. Afterwards, mucus dissolved in double volume of phosphate buffered saline (PBS, pH 7.4). Incubate the sample for 10-15 minutes at room temperature. Thereafter, add double the volume of ice cold acetone in mucus sample and incubated it for 45-60 minutes at 4 ºC. Centrifuge the sample at 10000 rpm for 10 minutes (4 ºC). Pellet taken and dissolved in PBS for determined the protein content using Nanodrop method.

ELISA

Indirect Elisa was performed using rubella vaccine (1:500 dilution) as coating antigen. Variable doses (156 – 1248µg) of mucin protein were used for the estimation of IgG antibody titre. Horse anti-serum used as secondary antibody and its optical density measured at 450 nm [15].

Total cellular content and proliferation assay

Infected human whole blood samples were collected from Mangal pathology lab, Baramati, District Pune, Maharashtra. In this study, human whole blood (100 µl) was taken in each falcon tube and then add serially diluted samples of mucin protein along with fixed concentration of rubella vaccine. Incubate the sample for 2 h at room temperature. After incubation, lysis (red cell lysis buffer) and washing (PBS, pH 7.4) the samples after centrifugation at speed (10000 rpm) for estimating total cellular content using Nanodrop [16].

Similarly, cytotoxicity assay were also performed using rubella vaccine. Incubate the samples of lysed human whole blood along with variable concentration of mucin protein in 96 well plate for 24 h incubation in carbon dioxide incubator. After incubation, centrifuge the samples at 2200 rpm for 4 ºC, 10 minutes. Discard supernatant and take pellet and dissolved in fresh medium. Again, incubate the samples for another 4 h along with MTT solution (5 mg/ml, 10 µl). Centrifuging the samples and discard supernatant. Fresh formazan crystals were appeared and settled at the bottom and then finally dissolved in dimethyl sulphoxide (DMSO) in a final volume of 0.2 ml. The optical density (OD) was measured at 570 nm [17].

Statistical analysis

The difference between control and variable doses of mucin protein is determined through one way ANOVA test (Bonferroni multiple comparison test).

RESULTS

Estimation of protein

The results of these studies claimed that mucus from *Macrochlymus indica* showedprotein content (10 µl, 1.401 mg/ml) which is determined through NanoDrop as shown in **Fig.1.**

ELISA

Indirect Elisa assay was performed using rubella vaccine as coating antigen as shown in **Fig.2**. The results showed that mucin proteinraised antibody production at lower doses as compared to rubella vaccine control. In other words, mucin protein from *Macrochlymus indica* could be a potent enhancer of B cells against rubella vaccine antigen.

Total cellular content

For these studies,total cellular content were also estimated in human whole blood along with variable concentration of mucin protein in presence of rubella vaccine as shown in **Fig.3**. The results showed that enhancement in total cellular content at lower doses as compared to rubella vaccine control.

Proliferation assay

The effect of variable concentration of mucin protein from *Macrochlymus indica* stimulated proliferative response in lysed human whole blood along with rubella vaccine as shown in **Fig.4**. At lower doses, there is enhancement in mucin protein proliferation as compared to rubella vaccine control. Overall, the data indicates that mucin protein from *Macrochlymus indica* stimulates T cell proliferation at lower doses.

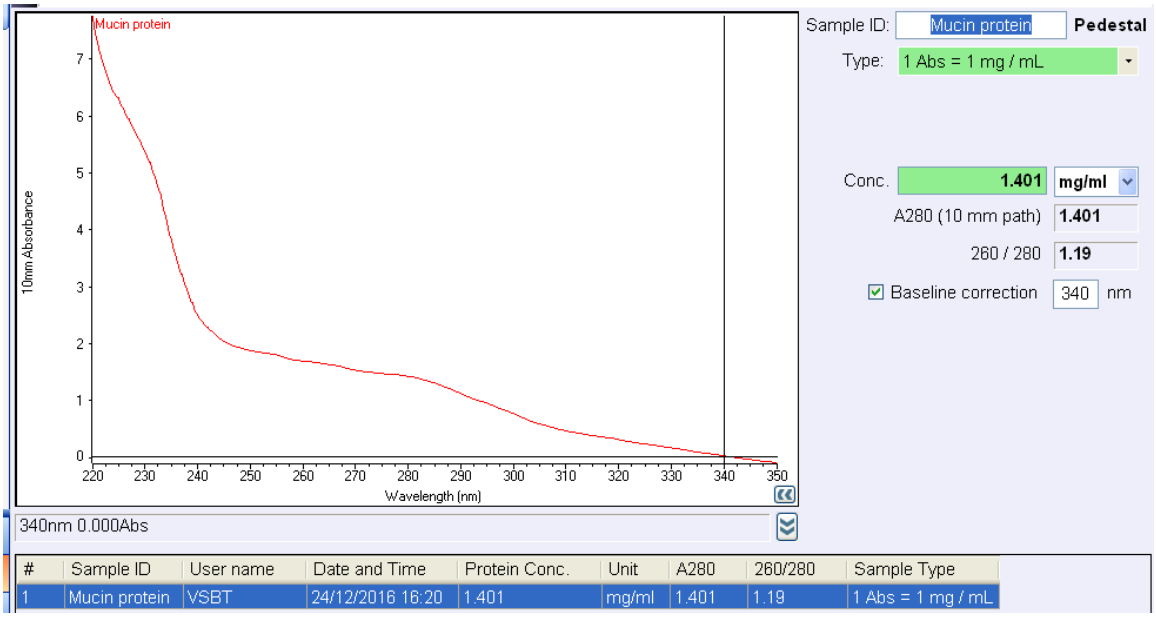
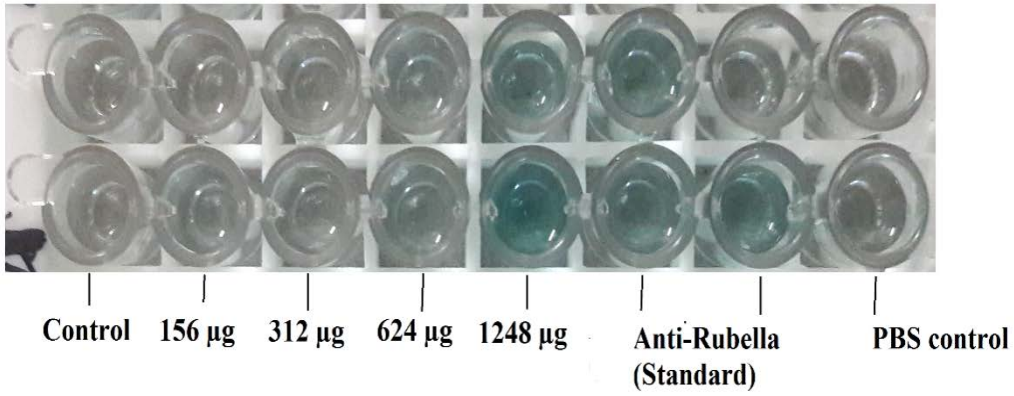


Figure 1: Estimation of protein content from mucus of *Macrochlymus indica* using Nanodrop method.

A)



B)

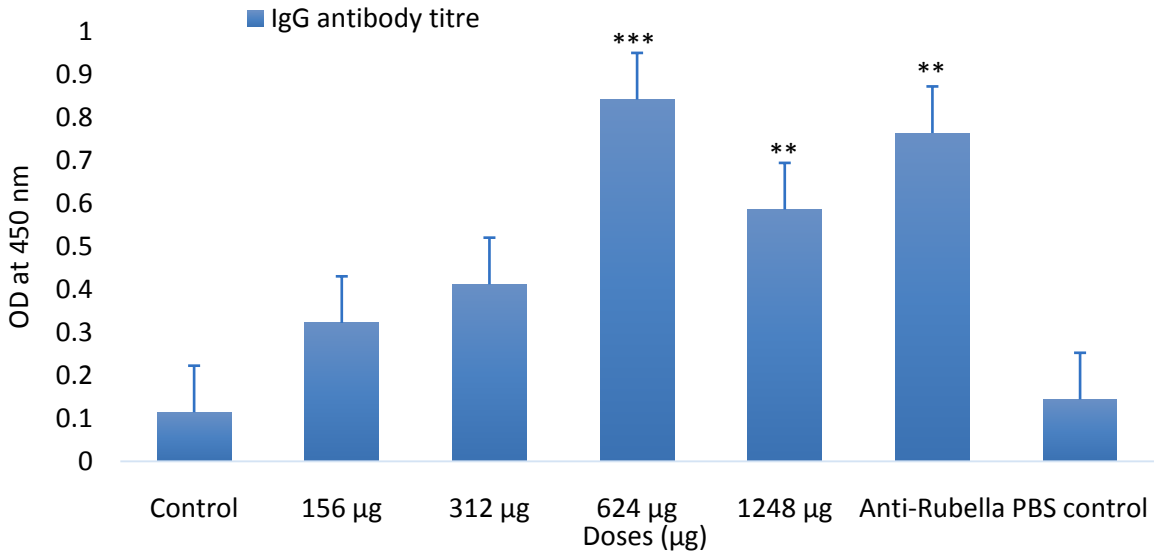
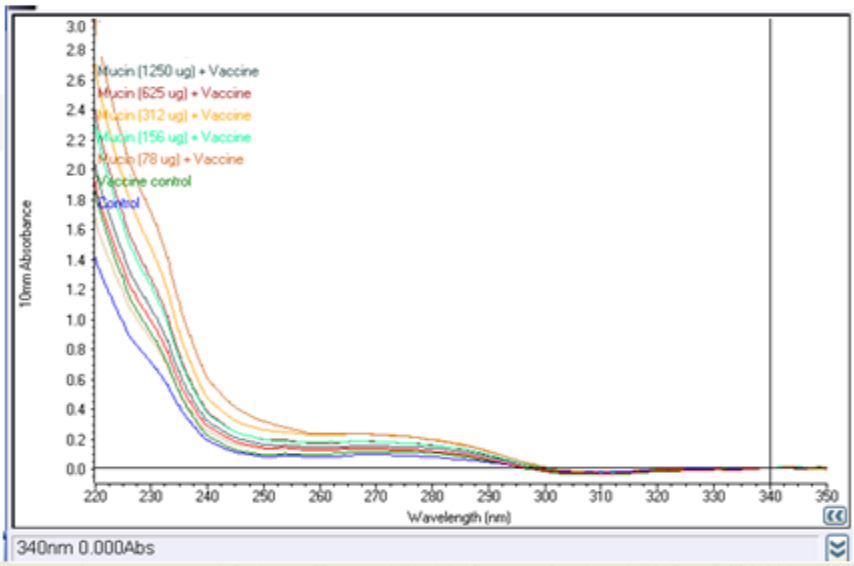


Figure 2: ELISA assay. Indirect ELISA was assayed using rubella vaccine as coating antigen using variable doses of protein from mucus of *Macrochlymus indica*for determining antibody titre. Horse anti-serum used as secondary antibody. The difference between control and variable doses of protein is determined through one way ANOVA test (Bonferroni multiple comparison test).
***P < 0.05; **P < 0.01 and ***P < 0.001**



Sample ID	User name	Date and Time	Protein Conc.	Unit	A280	260/280	Sample Type
Control	VSBT	06/01/2017 11:00	0.070	mg/ml	0.070	1.03	1 Abs = 1 mg / mL
Vaccine control	VSBT	06/01/2017 11:01	0.098	mg/ml	0.098	0.89	1 Abs = 1 mg / mL
Mucin (156 ug) + Vaccine	VSBT	06/01/2017 11:05	0.148	mg/ml	0.148	1.15	1 Abs = 1 mg / mL
Mucin (312 ug) + Vaccine	VSBT	06/01/2017 11:06	0.189	mg/ml	0.189	1.16	1 Abs = 1 mg / mL
Mucin (625 ug) + Vaccine	VSBT	06/01/2017 11:07	0.150	mg/ml	0.150	1.08	1 Abs = 1 mg / mL

Figure 3: Estimation of total cellular content in human whole blood. Lysed human whole blood were cultured with variable concentration of mucin protein extracted from *Macrochlymus indica*in presence of rubella vaccine. After incubation, lysis and washing (PBS, pH 7.4) the samples after centrifugation at speed (10000 rpm) for estimating total cellular content using Nanodrop method.

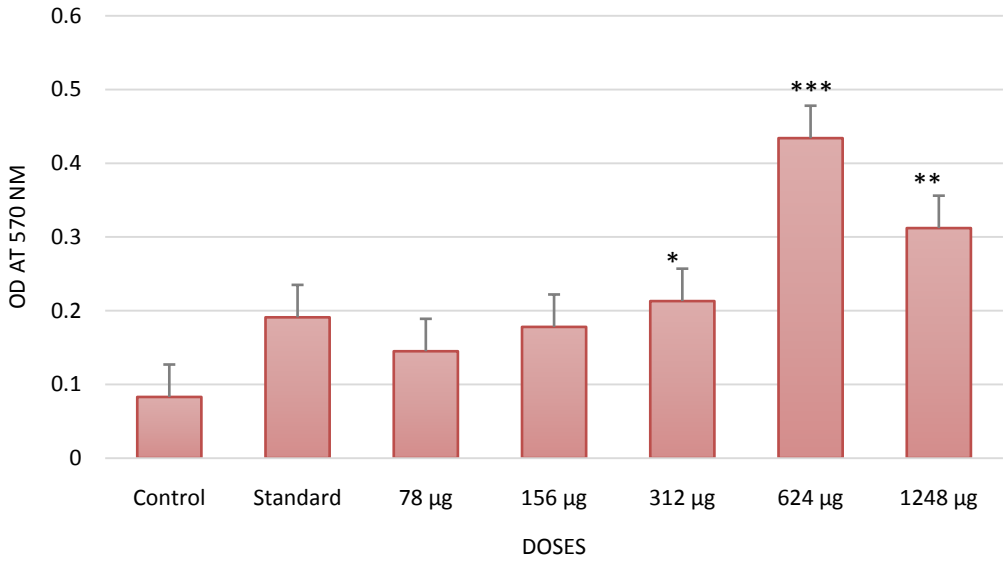


Figure 4: Proliferation assay. Lysed human whole blood were cultured with variable concentration of mucin protein extracted from *Macrochlymus indica*in presence of rubella vaccine. After incubation, centrifuge the samples and add MTT solution (5 mg/ml, 10 µl). Fresh formazan crystals were appeared and settled at the bottom and then finally dissolved in dimethyl sulphoxide (DMSO) in a final volume of 0.2 ml. The optical density (OD) was measured at 570 nm. The difference between control and variable doses of protein is determined through one way ANOVA test (Bonferroni multiple comparison test). *P < 0.05; **P < 0.01 and ***P < 0.001

DISCUSSION

One of the contagious diseases i.e. Rubella (also called German measles)viral disease is reported in human especially in case of pregnant women but its virus is totally different from Rubeola (regular measles). The major difference between these two viruses is that rubella, milder disease as compared to rubeola where it showed serious illness and caused serious complications. In contrast, rubella showed some adverse effect to pregnant women especially in the first 20 weeks of pregnancy period. If any pregnant women exposed to rubella definitely it may showed some defect in unborn baby or even miscarriage. For the last so many years, this rubella disease is neglected in India because of MMR vaccine (initiated in children; 13-18 months of age) and showed its protection against rubeola virus. The most common and observable marker is reported in measles i.e. Koplik spots where small red spots appeared on the mouth with blue white centers. As per the literature, U.S. epidemic is reported in between 1962–1965 where rubella virus infections were reported in pregnant women and estimated thousands of newly born baby were disabled. Now in US, rubella disease is totally eliminated in year 2004 [18]. In India, one of the study conducted by Sharma et al in Maharashtra and showed that pre-vaccination rubella immunity was still higher in the urban (80.2%) population as compared to the rural population (73.1%) [19, 20]. In this regard, we focused onmucin protein extracted from the mucus of*Macrochlymus indica* and determined its immunogencity potential against rubella vaccine. In the present study, mucin protein of *Macrochlymus indica* (Gastropoda family) wereused for these immunological based studies and these samples were collected in Vidya Pratishthan's garden. As per the literature, mucin protein of mucus extracted from Helix aspersa showed antibacterial activity against gram positive and gram negative bacteria. In addition, this species also showed anti-inflammatory activity in rat model studies. In view of this, another species of Gastropoda family were selected i.e. *Macrochlymus indica* and tried to determined its mucin protein content from mucus and determined its immunogenicity against rubella vaccine antigen.

Immunological finding of these studies related to mucin protein from *Macrochlymus indica* and showed significant increase in antibody titre at

lower doses in case of rubella vaccineas coating antigen which is determined through Indirect Elisa. In contrast, it also showed significant enhancement at lower doses intotal cellular content after incubation with variable doses of mucin protein along with rubella vaccine in lysed human whole blood.In short, lower doses of mucin protein showed immunostimulatory activity but at higher doses, it showed dose dependent reduction in rubella vaccine proliferation. This immunostimulatory effect of mucin protein inducingproliferation rate including total cellular content and antibody production at lower doseswhich indicates that mucin proteinstimulated cell mediated immunity. These results suggest that mucin protein may stimulate cellular immune response against rubella vaccine antigen. Overall, these results suggest its immunogenicity potential of mucin protein from *Macrochlymus indica* against rubella vaccine antigen.

CONCLUSION

The results obtained in this immunological study which clearly indicates that mucin protein from *Macrochlymus indica* have significant immunogenicity properties. In this study, variable concentration of mucin protein wasfound to be more potent because of higher amount of antibody production and proliferation rate against rubella vaccine at lower doses. Further studies were conducted in order to analyzed HPLC analysis of mucin protein and also conducted anti-bacterial activity against gram positive and gram negative bacteria.

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